Data Evaluation Record on the Acute Toxicity of BAS 500 00 F (AI: Pyraclostrobin) to Fish, *Oncorhynchus mykiss*

PMRA Submission Number {.....} EPA MRID Number 49604102

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	427266
	OECD Data Point	{.....}
	EPA MRID	49604102
	EPA Guideline	850.1075

Test material: **BAS 500 00 F** **Purity:** 24%
Common name: Pyraclostrobin
Chemical name: IUPAC methyl N- (2- {[1- (4- chlorophenyl)- 1H- pyrazol- 3- yl]oxymethyl} phenyl)-(N-methoxy)carbamate
CAS name
CAS No. 175013-18-0
Synonyms: None Reported

Primary Reviewer: Moncie Wright **Signature:**

Moncie V Wright

Environmental Scientist, CDM Smith, Inc. **Date:** 7/24/15

Secondary Reviewer: John Marton, Ph.D. **Signature:**
Environmental Scientist, CDM Smith, Inc. **Date:** 11/09/15

Reviewer: Lewis R. Brown, Env. Biologist **Date:** 01/12/16
EPA/OCSPP/OPP/EFED/ERB-1

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	099100	

Date Evaluation Completed: 12-01-16

CITATION: Salinas, E. 2014. BAS 500 00 F: Acute Toxicity Study in the Rainbow Trout (*Oncorhynchus mykiss*). Study conducted by BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany. Laboratory Project No.: 18F0262/03E009. Study sponsored by BASF SE, Ludwigshafen, Germany. Study initiated July 14, 2014 and completed December 18, 2014

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the

conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to **BAS 500 00 F (AI: Pyraclostrobin)** at nominal concentrations of 0 (control), 0.0025, 0.0040, 0.0064, 0.01024, and 0.0164 mg pyraclostrobin/L under flow-through conditions. The reviewer-calculated mean-measured concentrations were <LOQ (<0.00024, control), 0.00255, 0.00390, 0.00617, 0.0100, and 0.0164 mg pyraclostrobin/L.

The 96-h LC_{50} was 0.00732 mg ai/L. Sublethal effects (tottering) were observed in the groups exposed to 0.0100 mg ai/L and 0.0164 mg ai/L

Based on the results of this study, **BAS 500 00 F (AI: Pyraclostrobin)** would be classified as very highly toxic to *Oncorhynchus mykiss* in accordance with the classification system of the U.S. EPA.

This study **is scientifically sound** and is classified as **Acceptable**.

Results Synopsis

Test Organism Size/Age (mean weight or length): 4 months old; 2.05 g, 6.2 cm
Test Type (Flow-through, Static, Static Renewal): Flow-through

LC_{50}: 0.00732 mg ai/L 95% C.I.: 0.00679 – 0.00789 mg ai/L
Probit Slope: N/A 95% C.I.: N/A

Endpoint(s) Affected: mortality and sub-lethal effects including: tottering

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted according to the Commission Regulation (EC) No. 440/2008, Part C.1, OECD Guideline No. 203 (1992), U.S. EPA 540/09-82-024, § 72-1 (1982), and U.S. EPA OPPTS 850.1075 (Public Draft, 1996). The reviewer assessed the study methods and results according to U.S. EPA OPPTS 850.1075 and OECD 203, and noted any similarities and/or differences. A couple of deviations were noted:

1. The study author did not determine the concentrations of particulate matter, metals, pesticides, boron, fluoride, or chlorine in the dilution water; EPA OPPTS guidance suggests that chemical analysis of water used in testing should include those elements. However, OECD guidance does not suggest that chemical analysis of water should include those measurements.
2. The study author did not implement a 15 to 30 minute transition period between light and dark conditions as suggested by OPPTS guidance. However, OECD guidance does not address the need for a transition period.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act), which meet the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and the Principles/Standards of FIFRA and TSCA.

A. MATERIALS:

1. Test material **BAS 500 00 F (AI: Pyraclostrobin)**

Description: Clear brown liquid

Lot No./Batch No. : 590-86

Purity: 24%

Stability of compound under test conditions: Analytical verification of the test material yielded measured concentrations that were ≥93% of the nominal test concentrations throughout the test period.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored at ambient temperature.

Physicochemical properties of BAS 500 00 F (AI: Pyraclostrobin).

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: 4 months old

Weight at study initiation: 2.05 g (1.62 – 2.61 g)
* Determined at study termination using all surviving fish in the control.
EPA recommends: mean 0.5 - 5 g.

Length at study initiation: 6.2 cm (5.6 – 6.6 cm)
* Determined at study termination using all surviving fish in the control.

EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∀ 1.0 cm for bluegill and 5.0 ∀ 1.0 cm for rainbow trout

Source: Fish were obtained from Forellenzucht Trostadt GbR, Trostadt, Germany.
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was not conducted.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		
Period:	At least 14 days.	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Conditions: (same as test or not)	Same as test (dilution water, temperature, and light regime).	
Feeding:	Fish were fed Inicio 917 (Bio Mar, Denmark) *ad libitum*, and were additionally fed frozen brine shrimp (*Artemia*) on workdays.	
Health: (any mortality observed)	No medical treatment was provided during acclimatization, and mortality was 0.8% during the last week prior to test initiation.	
Duration of the test	96 hours	*The recommended test duration is 96 hours.*
Test condition		The flow rate provided approximately 6 volume additions every 24 hours.
Static/flow-through	Flow-through	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Type of dilution system - for flow-through method.	Not reported	
Renewal rate for static renewal	N/A	
Aeration, if any	No aeration was provided during the test.	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Stainless steel 9 L 9 L	 *Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Non-chlorinated charcoal filtered drinking water (Frankenthal, Germany) mixed with deionized water and aerated before sanitization by UV treatment prior to entering the aquaria.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency=s 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf)* *Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 100 mg/L $CaCO_3$ 7.8 – 8.0 7.9 – 9.8 mg/L (75% of the maximum saturation at the test temperature of 14°C is 7.78 mg/L) <2 mg/L (1.2 mg/L in the water supply at the start of the exposure) Not reported Not reported Not reported Not reported 13.0 – 14.1°C N/A Temperature, dissolved oxygen, and pH were measured daily.	Conductivity: 250 µS/cm (258 µS/cm as measured in the water supply at the start of the exposure) *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: >60% during first 48 hrs and > 40% during second 48 hrs; flow-through: >60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 °C for coldwater species, 17 or 22 °C for warmwater species, and 22 ± 1 °C for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 2 N/A 2	*Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 N/A 10	*Number of organisms per replicate should be ∃10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.38 g fish tissue/L/day	*Recommended static conditions are # 0.8 g/L at # 17EC and # 0.5 g/L at > 17EC. Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: Nominal: Mean-measured:	0 (control), 0.0098, 0.0156, 0.025, 0.040, and 0.064 mg BAS 500 00 F/L 0 (control), 0.0025, 0.0040, 0.0064, 0.01024, and 0.0164 mg pyraclostrobin/L <LOQ (<0.001, control), 0.00981, 0.0150, 0.0237, 0.0386, and 0.0630 BAS 500 00 F/L <LOQ (<0.00024, control), 0.00255, 0.00390, 0.00617, 0.0100, and 0.0164 mg pyraclostrobin/L	
Solvent (type, percentage, if used)	N/A- no solvent was used	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D 76-675 lux light intensity	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed 48 hours prior to or during the test.	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination	Samples collected at time 0, 48 hours, and at 96 hours were analytically determined for the concentration of the test material via HPLC-MS.	

Parameter	Details	Remarks *Criteria*
Level of quantization Level of detection	0.001 mg BAS 500 00 F/L; 0.00024 mg pyraclostrobin/L Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and signs of toxicity (changes in appearance and abnormal behavior)	
Observation intervals	1, 6, 24, 48, 72, and 96 hours of exposure	 *Observation intervals should be a minimum of every 24 hours.*
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

There was no mortality in the control or the mean-measured 0.00255 and 0.00390 mg ai/L treatment levels. Mortality was 15, 100, and 100% in the 0.00617, 0.0100, and 0.0164 mg ai/L treatment levels, respectively.

Table 3: Effect of BAS 500 00 F (AI: pyraclostrobin) on Mortality of *Oncorhynchus mykiss*.

Treatment (mg ai/L) Mean-measured (and nominal)	No. of fish at start of study	Observation period					
		Day 1		Day 2		Day 4	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control (dilution water only)	20	0	0	0	0	0	0
0.00255 (0.0025)	20	0	0	0	0	0	0
0.00390 (0.0040)	20	0	0	0	0	0	0
0.00617 (0.0064)	20	0	0	1	5	3	15
0.0100 (0.010)	20	2	10	15	75	20	100
0.0164 (0.016)	20	20	100	20	100	20	100
NOAEC	0.00406 mg ai/L (based on nominal concentrations, converted by the reviewer)						
LC_{50}	0.0070 mg ai/L (based on nominal concentrations, converted by the reviewer) 0.0068 mg ai/L (based on mean-measured concentrations, converted by the reviewer)						
Positive control, if used mortality: LC_{50}:	N/A						

B. NON-LETHAL TOXICITY ENDPOINTS:

No sublethal effects were observed in the control or any of the treatment groups tested except the mean-measured 0.0100 mg ai/L and 0.0164 mg ai/L treatment level. At 6 hours, 65% of fish were tottering in the 0.0164 mg ai/L test level. By 24 hours, all fish in this test group were dead. At 24 and 48 hours, 72 and 80% of fish were tottering, respectively. By test termination, all fish were dead.

Table 4: Sub-lethal Effect of BAS 500 00 F (AI: pyraclostrobin) on *Oncorhynchus mykiss*.

Treatment (mg ai/L) Mean-measured (and nominal)	Observation period		
	Day 1	Day 2	Day 4
	% affected	% affected	% affected
Control (dilution water only)	0	0	0
0.00255 (0.0025)	0	0	0
0.00390 (0.0040)	0	0	0
0.00617 (0.0064)	0	0	0
0.0100 (0.010)	T- 72%	T- 80%	All fish were dead
0.0164 (0.016)	All fish were dead	All fish were dead	All fish were dead
NOAEC	0.0040 mg ai/L (based on nominal concentrations)		
LOAEC	0.0064 mg ai/L (based on nominal concentrations)		
EC_{50}	N/A		
Positive control, if used % sublethal effect: EC_{50}:	N/A		

T- tottering

C. REPORTED STATISTICS:

The study author statistically evaluated the data and calculated an LC_{50} value using the probit method (Finney, 1971) via the commercial software program TOXRAT Professional 2.10 (ToxRat Solutions GmbH, Alsdorf, Germany).

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer conducted the statistical analysis using the program CETIS (version 1.8.7.12) with database backend settings implemented by EFED on 3/25/14 and the reviewer-calculated mean-measured concentrations. The probit method was attempted, but an LC_{50} value could not be calculated. The results of the untrimmed Spearman-Karber test were reported by the reviewer.

LC_{50}: 0.00732 mg ai/L 95% C.I.: 0.00679 – 0.00789 mg ai/L
Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in general agreement; there was treatment-related toxicity in this study. The study author used the probit method, but this method was not suited to the study data and 95%

confidence intervals could not be calculated. The reviewer used the untrimmed Spearman-Karber to calculate the LC_{50} value its accompanying 95% confidence limits. The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The study author used the % purity and the density of the active ingredient to convert the nominal concentrations of the formulation to nominal concentrations of the active ingredient. The reviewer calculated a conversion factor to convert the study author-provided measured concentrations of the formulation to the measured concentrations of the active ingredient pyraclostrobin.

The in-life portion of this study was conducted from August 11 to 15, 2014.

G. CONCLUSIONS:

This study **is scientifically sound** and is classified as **Acceptable**. There was treatment-related mortality and sublethal effects in this study. Using mean-measured concentrations and the untrimmed Spearman-Karber test, the reviewer calculated an LC_{50} value of 0.00732 mg ai/L.

LC_{50}: 0.00732 mg ai/L 95% C.I.: 0.00679 – 0.00789 mg ai/L
Probit Slope: N/A 95% C.I.: N/A

III. REFERENCES:

Kahl MD, Russom CL, DeFoe DL, Hammermeister DE. 1999. Saturation units for use in aquatic bioassays. Chemosphere 39(3):539-551.